Sucrose concentration alters fermentation kinetics, products, and carbon fates during in vitro fermentation with mixed ruminal microbes

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Sucrose concentration alters fermentation kinetics, products, and carbon fates during in vitro fermentation with mixed ruminal microbes[1]

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ABSTRACT: Effects of sucrose (Suc) concentration on fermentation kinetics and products were evaluated using 3 concentrations of Suc, with 1 concentration of isolated NDF from Bermudagrass fermented together in batch culture in vitro with rumen inoculum. Fixed amounts of medium and inoculum were the protein sources, so protein:Suc decreased with increasing Suc. Kinetics were calculated from gas production over 48 h in a randomized complete block design (n = 28), and product yield was evaluated with sampling every 4 h for 24 h in a split-split plot in time design (n = 84). Fermentation vial was the experimental unit. Increasing Suc increased the lag time of rapidly ($P < 0.01$) and slowly fermented ($P < 0.01$) fractions and tended to decrease the rate of gas production from the rapid fraction ($P = 0.07$). Gas production from the slow fraction decreased linearly with increasing Suc ($P = 0.02$), suggesting a decrease in NDF fermentation. Sucrose was the predominant substrate at ≤8 h of fermentation. Maxima for microbial CP (MCP) production were detected at ≤8 h of fermentation. At detected MCP maxima, MCP production increased linearly ($P = 0.02$) and total organic acids (sum of lactate, acetate, propionate, and butyrate; mmol) tended to increase linearly ($P = 0.07$) with increasing Suc. Maximum lactate production at 0 and 4 h increased ($P = 0.01$), and yield of lactate from Suc tended to increase, linearly ($P = 0.09$) with increasing Suc. At detected MCP maxima, yield of C in products (total organic acids, MCP, CO_2, CH_4, glycogen) from utilized Suc C declined linearly for total products ($P = 0.01$) and organic acids ($P = 0.01$) and tended to decline for MCP ($P = 0.12$) as Suc increased. This may be a function of increased catabolic inefficiency of microbes with increasing Suc, as evidenced by increasing yields of lactate, or the use of C for products not measured. Product C yields were 1.28, 0.98, and 0.81 from lowest to greatest Suc inclusion, respectively. Values >1 indicate incorporation of C from the medium, likely from AA and peptides. The results support the premises that direct effects of Suc concentration and perhaps protein:Suc alter yields of fermentation products. That substrate concentration altered fermentation products and kinetics, possibly due to interactions with the run conditions, advises the clear definition of substrates and fermentation conditions to determine how the results integrate into our knowledge of ruminant nutrition.

Key words: cattle, fermentation kinetics, fermentation product, nonfiber carbohydrate, rumen fermentation, sucrose

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INTRODUCTION

The mass amounts of fermentation products produced per unit of substrate fermented need to be estimated to describe the nutritional value of a ruminally fermented substrate to the host animal, or to predict the production of fermentation products such as greenhouse gases for estimates of environmental impact. This requires knowledge of the direct effects and interactions of factors that alter digestion kinetics of the substrate or that modify partitioning of substrate to different products. When in vitro fermentations are used to make these measures, corrections must be made for quantities of analytes not derived from the substrates or that do not function similarly in treatments and fermentation blanks.

Effects of changing the concentration of a carbohydrate substrate on fermentation kinetics and on products produced by mixed ruminal microbes have not been explored systematically. Further, the research literature lacks comprehensive descriptions of the mass conversion of substrate to fermentation products. Mass transformations in the rumen are affected by many

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factors, one of which is nutrient availability (Russell and Sniffen, 1984; Stokes et al., 1991). Type and concentration of nutrients are known to affect rate and extent of substrate consumption, fermentation product distribution, and yield of microbial cells. Studies have tended to report on production or yield of several fermentation products but have not attempted to determine the extent to which products account for the mass of the original substrate.

The objectives of these experiments were to describe the effect of different concentrations of sucrose on yield and allocation of substrate C to fermentation products at the detected maxima of microbial production from sucrose and on digestion kinetics. Methods for correcting treatment values for fermentation products in fermentation blanks or for those introduced by the medium and inoculum were also evaluated.

MATERIALS AND METHODS

Sample Preparation and Fermentation Substrates

Isolated Bermudagrass (*Cynodon dactylon,* L.) NDF (**isolNDF**) prepared from Bermudagrass hay ground to pass the 1-mm screen of a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). Hay (50 g) was refluxed with 2 L of neutral detergent solution (Mertens, 2002) with 10 mL of heat-stable α-amylase (Termamyl 120L, Novo Nordisk Biochem, Franklinton, NC) for 1 h. The residue was filtered through a 37-μm pore size nylon cloth with repeated rinsing with boiling distilled water and then held overnight at room temperature in 1 *M* ammonium sulfate solution (approximately 5 g of residue DM/200 mL of solution) to remove residual detergent. The residue was then filtered under vacuum through the nylon cloth and rinsed with boiling distilled water until no additional foaming from the detergent was visible. The residue was rinsed twice with acetone and filtered under vacuum until dry. After further drying overnight in a 55°C forced-air oven, the residue was allowed to equilibrate to ambient humidity. The isolNDF was analyzed for DM (96.6%), OM (100% of DM), CP (4.59% of DM; AOAC, 1980), and NDF (99.9% of DM) using heat-stable α-amylase (Termamyl 120L, Novo Nordisk Biochem) but no sodium sulfite (Mertens, 2002).

Fermentation substrates were the treatments in this study. Substrates for fermentation product analysis consisted of 130 mg of isolNDF with sucrose (99+%, ACS reagent, Aldrich Chemical Co. Inc., Milwaukee, WI) added at 65 (**Suc65**), 130 (**Suc130**), or 195 (**Suc195**) mg and weighed individually into 50-mL serum vials (6 replicates each per fermentation time point). Due to space limitations in the water bath, a treatment of isolNDF alone was not included. For the fermentation to measure gas production, substrates included 0, 20, 40, or 60 mg of sucrose and 40 mg of isolNDF per vial (6 replicates each) and 4 vials containing only 60 mg of sucrose (no isolNDF) as a substrate. Fermentation blanks containing no substrate were included for each sampling time.

Experiment 1. Gas Production and Kinetics

Samples were fermented in sealed serum vials for the estimation of gas production, rate of fermentation, and lag time for rapid and slow pools for 48 h according to the methods of Weimer et al. (2000). Gas production in each sealed vial (50 mL nominal volume) was continuously monitored using pressure sensors linked to a computerized recording system, as described by Mertens and Weimer (1998). A single fermentation run was performed using Goering and Van Soest (1970) medium and blended inoculum (total of 10 mL medium + inoculum). Medium in each vessel provided 1.7 mg of CP from NPN and 3.5 mg of CP from true protein (including 0.37 mg of N from the cysteine-HCl added as a reducing agent). Contents of the fermentation vessels were analyzed for organic acids as described below and for residual NDF. Residual NDF was analyzed by the method of Mertens (2002) using 20 mL of neutral detergent but without amylase or sodium sulfite. Serum vial contents were quantitatively transferred to Berzelius beakers, with rinses of the vial using neutral detergent. Values for NDF are expressed on a DM basis, with no correction for ash.

Experiment 2. Fermentation Product Analysis

Replicate vials containing substrates and fermentation blanks were prepared for each of 7 sampling times, at 0, 4, 8, 12, 16, 20, and 24 h, giving a total of 168 vials per fermentation run. The vials were flushed continuously with CO_2 from the time that the medium was added to the time that the vials were sealed. Two replicate fermentation runs were performed.

The 2 ruminally cannulated lactating Holstein cows used as inoculum donors were maintained under protocols approved by the University of Wisconsin Institutional Animal Care and Use Committee. Rumen inoculum was prepared by combining 500 mL of rumen fluid filtered through 4 layers of cheesecloth with the liquid from 500 mL of medium that had been mixed in a blender with 250 g of rumen solids and similarly filtered through cheesecloth. All inocula were held under CO_2 in a 39°C water bath until inoculation. The donor cows were fed a total mixed ration consisting of, on a DM basis, 30% corn grain, 30% corn silage, 30% alfalfa haylage, and 10% soybean meal, with supplemental vitamins and minerals to meet the NRC (2001) recommendations.

Medium (21.4 mL), reducing solution (1.0 mL), and blended rumen inoculum (9.6 mL) were added to each 50-mL serum vial, which was then sealed with a butyl rubber stopper and an aluminum crimp seal, and incubated in a water bath at 39°C. The medium and reducing solution provided 62.3 mg of an enzymatic digest of casein, 7.9 mg of cysteine-HCl, and 24.9 mg of ammonium bicarbonate, for a total of approximately 84.6 mg of CP per vial (57.0 mg from amino-N sources, 27.6 mg from NPN sources). Theoretically, when compared with

the amount of microbial CP measured in this experiment, available N should not have limited microbial growth.

At each sampling time, fermentation vials for that hour were removed from the water bath and immediately placed in an ice bath. Samples at 0 h were inoculated on the bench and placed in an ice bath within 0.5 min. For the 6 vials from each treatment, the pH of 2 vials was recorded and their contents were frozen at –20°C for later analysis of the supernatant for organic acids and residual sucrose, glucose, and fructose. Two vials were frozen at –20°C, subsequently lyophilized, and the entire contents of the vials were analyzed for glycogen. The remaining 2 vials were used to determine microbial CP (**MCP**) as trichloroacetic acid (**TCA**)-precipitated CP (**TCACP**).

To the vials used for MCP determination, 6.4 mL of 120% (wt/vol) TCA was added to each vial to achieve a final concentration of 20% TCA. The TCA was added in increments to minimize foaming. Each vial was then placed in an ice bath for 45 min, after which a vial's contents were quantitatively transferred to two 50-mL polyethylene centrifuge tubes using minimal amounts of 20% TCA to rinse the vials. Samples were centrifuged at 7,719 × *g* at 5°C for 20 min. Contents of each pair of centrifuge tubes were quantitatively transferred into a single Whatman 541 filter paper (Fisher Scientific) and allowed to filter by gravity. Centrifuge tubes and residues in the filter paper were rinsed using chilled 5% TCA. The filtrate was then filtered through a Whatman GF/A glass fiber filter (Fisher Scientific), using 5% TCA to rinse the flask and the residue; the second filtrate was discarded. Both filters, representing the contents of a single fermentation vial, were placed into labeled sample boats for N analysis.

Fermentation Residue and Medium Analysis

For estimation of MCP, the TCA-precipitated samples in Whatman 541 and GF/A filters, representing a single fermentation tube, were analyzed separately for N (Dumas combustion method; Leco FP-2000, Leco Corporation, St. Joseph, MI), and their results were summed. The CP content of TCA-precipitated samples was calculated as N content × 6.25. The amount of TCACP in each fermentation vial was corrected for the average value from the 0 h for that treatment, which accounted for TCACP contributed by medium, inoculum, and substrate. The corrected TCACP values that are reported and used in all calculations represent a conservative estimate of MCP production because they assume no conversion of sample 0-h TCACP to MCP.

Organic acid concentrations in samples of medium were analyzed by HPLC (Weimer et al., 1991). Residual sucrose, glucose, and fructose in media were analyzed by HPLC, with residual sucrose estimated as sucrose + [0.95 × (glucose + fructose)]. The HPLC for analyzing residual sucrose was equipped with an anion exchange analytical column (CarboPac PA1, Dionex, Sunnyvale, CA), the mobile phase was 100 m*M* NaOH, the flow rate was 1.0 mL/min, and the injection volume was 10 μL. In this system, the carbohydrates were detected by pulsed amperometric detection. Values for organic acids and residual sugars were corrected for the average of the fermentation blanks for the sampling hour. Total organic acid values reported are the sum of acetate, propionate, butyrate, and lactate. Total VFA values are the sum of acetate, propionate, and butyrate and were reported for Exp. 1 because no lactate was detected at the 48-h time of measurement.

Lyophilized samples were analyzed for glycogen after lysis of microbial cells with 20 mL of 0.2 *M* NaOH at 100°C for 15 min. Samples were filtered through glass wool into 100-mL volumetric flasks and brought to volume with distilled water. In duplicate test tubes, 1 mL of sample was added to 1 mL of 0.1 *M* sodium acetate buffer (pH 4.5) and 50 μL of amyloglucosidase (~7 units; Sigma A3514, Sigma-Aldrich Co., St. Louis, MO), vortexed, and incubated at 60°C for 45 min with periodic swirling of the tubes. To one of the duplicate tubes, 1 mL of distilled water was added to dilute the sample, and the tube was vortexed. From each incubated sample, 0.5-mL portions were transferred to duplicate test tubes for measurement of released glucose (Karkalas, 1985). Glycogen was calculated as glucose × 0.9. Glycogen values were corrected for the average value of the fermentation blank at 0 h.

Production of methane and carbon dioxide were estimated from organic acid production according to the equations of Hungate (1966). These equations presume that no organic acid was produced from substrates other than carbohydrate. These equations were also used to estimate hexose fermented in fermentation blanks between specific time points using VFA values calculated by subtracting the values for the earlier hour from the later hour. Potentially fermentable material in the fermentation blanks was calculated by subtracting the 0-h values for VFA from the 24-h values.

It was not possible to perform all analyses on each individual fermentation vial, so the values used to estimate yields were averaged to provide 1 set of values for each analyte per treatment per incubation hour in each fermentation. Yield of lactate from utilized sucrose was calculated as: mmol of lactate/(mg of sucrose – unfermented sucrose); unfermented sucrose was calculated as 0.95 × (residual glucose + fructose in medium).

Carbon in microbial products and substrates was calculated in mg as 0.51 mg of C/mg of MCP, 0.44 mg of C/mg of glycogen, 0.40 mg of C/mg of glucose, 0.40 mg of C/mg of fructose, 12 mg of C/mmol of CO_2, 12 mg of C/mmol of CH_4, 24 mg of C/mmol of acetate, 36 mg of C/mmol of propionate, 48 mg of C/mmol of butyrate, and 36 mg of C/mmol of lactate. Total C in the products was the sum of MCP, glycogen, CO_2, CH_4, and organic acids. Sucrose C that was utilized equaled: (mg of sucrose × 0.421) – mg of C in residual glucose and fructose.

Statistical Analysis

Variables used in the analyses were as follows: Y = the dependent variable, μ = the overall mean, R_i = the fermentation run (i = 1, 2), S_j = the sucrose amount (j = 65, 130, or 195 mg in Exp. 2, or 0, 20, 40, or 60 mg for Exp. 1), H_k = the sampling hour (k = 0, 4, 8, 12, 16, 20, 24), all interaction terms, and ε = residual error.

Except where noted, all analyses were performed using the MIXED procedure (SAS Inst. Inc., Cary, NC). In the MIXED models, fermentation run and all interaction terms containing it were treated as random variables. Values are reported as least squares means with SEM or SED.

Experiment 1. Gas production and kinetic data were evaluated using the GLM procedure of SAS with the model $Y_j = \mu + S_j + \varepsilon_j$. Evaluation of the rapidly fermenting pool did not include data from isolNDF fermented alone. Sucrose amount was treated as a classification variable in models used to evaluate linear and quadratic effects of sucrose inclusion level using orthogonal contrasts and to calculate least squares means and SEM. Because the slowly fermented fraction had 4 levels of sucrose inclusion (0, 20, 40, or 60 mg/vial), the cubic effect of sucrose on this fraction was also evaluated using orthogonal contrasts, but it was not significant ($P > 0.62$) for any measure and is not presented. The model above was also used to derive statistics (R^2 and root mean square error) to describe the fit of the linear and quadratic regression equations of the sucrose effect, but with sucrose amount as a continuous variable; a quadratic term for sucrose amount was included when quadratic equations were evaluated. The values for fit of the regression lines are included particularly for use by those interested in using the data in mathematical models.

Evaluation of the additivity of gas production from sucrose and isolNDF fermentation was performed as a paired *t*-test. The 48-h gas production values for 60 mg of sucrose + 40 mg of isolNDF fermented together were compared with values calculated as the sum of milliliters of gas produced/milligrams of substrate fermented from the separate fermentations of 60 mg of sucrose and 40 mg of isolNDF. Sucrose was assumed to be entirely fermented by 48 h, and isolNDF substrate was corrected for residual NDF measured at the end of the 48-h fermentation.

Experiment 2. Medium content of glucose, fructose, glycogen, and lactate at the time of their detected maxima, and MCP, glycogen, organic acids, fermentation gases, and C in microbial products at time of detected MCP maxima were compared among sucrose treatments. Although sampling at 4-h intervals made it unlikely that the precise time and amount of maximal production of glycogen, lactate, or MCP were detected, these values allowed evaluation of relative differences among treatments. The data were evaluated using type III sums of squares and Suc treatment as a classification variable with the model $Y_{ij} = \mu + R_i + S_j + RS_{ij} + \varepsilon_{ij}$. Orthogonal contrasts were used to determine linear or quadratic effects of sucrose treatment.

The temporal patterns of MCP decay curves after peak MCP production were evaluated with the model $Y_{ijk} = \mu + R_i + S_j + RS_{ij} + H_k + RH_{ik} + SH_{jk} + RSH_{ijk} + \varepsilon_{ijk}$. All variables were treated as classification variables in initial analyses performed using the MIXED procedure of SAS. In subsequent analyses using the GLM procedure of SAS, linear and quadratic terms for hour and associated interaction terms were tested, with the hour terms treated as continuous variables. Data from each sucrose treatment and fermentation blanks were analyzed separately in these analyses.

RESULTS AND DISCUSSION

Experiment 1. Gas Production and Fermentation Kinetics

The 48-h gas production values for 60 mg of sucrose and 40 mg of isolNDF fermented together (arithmetic mean 30.6 mL, SEM = 0.16) or fermented separately and their values summed (arithmetic mean 30.3 mL, SEM = 0.16) did not differ ($P = 0.31$), indicating that gas production from the fermentation of sucrose and isolNDF were additive. A linear increase in lag time ($P < 0.01$) but no change in rate of fermentation ($P \geq 0.87$) was noted for the slowly fermenting fraction as sucrose inclusion increased (Table 1). An increase in lag time could affect end point measures of NDF before fiber achieved its full extent of fermentation. Gas production from the slowly fermenting fraction decreased linearly with increasing amounts of sucrose ($P = 0.02$). Although sucrose has been reported to suppress fiber disappearance from the rumen and the enzymatic activities of fiber-associated bacteria (Huhtanen and Khalili, 1992), the near instantaneous disappearance of sucrose at the rates reported for its hydrolysis by ruminal microbes (~1,400%/h; Weisbjerg et al., 1998) makes sucrose itself an unlikely cause of the prolonged lag time. The potential availability of glucose from hydrolyzed sucrose in the early hours of the fermentation could have delayed the onset of growth, enzyme synthesis, or enzyme activity by the fibrolytic bacteria; however, fibrolytic enzymes of anaerobic bacteria are generally resistant to repression or inhibition by glucose (Lynd et al., 2002). Piwonka and Firkins (1996) demonstrated that fermentation products from glucose could reduce the fermentation of fiber by mixed ruminal microbes in vitro even when low pH was not a factor. With no detected difference among treatments in the proportion of NDF fermented by 48 h ($P \geq 0.25$; Table 2), the addition of sucrose would have to have modified the yield or profile of fermentation products from the slow fraction to change gas production. Alternatively, it is possible that gas production is a more sensitive tool for detecting differences in carbohydrate fermentation than is measurement of small quantities (9 to 10 mg) of unfermented NDF, including that reduced to very fine parti-

Table 1. Fermentation kinetics data based on gas production measurements from the fermentation of sucrose and isolated NDF (Exp. 1)[1]

Item	Substrate[2]				SEM	*P*-value, sucrose effect		Linear regression descriptors[4]	
	isolNDF	Suc20	Suc40	Suc60		Linear	Quadratic[3]	R^2	RMSE
pH at 48 h	6.65	6.48	6.26	6.04	0.01	<0.01	<0.01	—	—
Rapidly fermented fraction									
Total gas, mL	—	8.04	15.89	22.73	0.30	<0.01	0.19	0.99	0.745
kd,[5] h^{-1}	—	0.335	0.292	0.286	0.017	0.07	0.41	0.20	0.0425
Lag, h	—	0.02	0.26	1.14	0.06	<0.01	0.53	0.91	0.155
Slowly fermented fraction									
Total gas, mL	9.68	9.92	9.54	7.53	0.61	0.02	0.08	0.21	1.541
kd,[5] h^{-1}	0.053	0.053	0.054	0.053	0.005	0.97	0.87	—	—
Lag, h	4.31	7.95	10.37	12.96	0.62	<0.01	0.41	0.83	1.474

[1]Values presented as least squares means.
[2]isolNDF = isolated NDF alone, Suc20 = isolNDF + 20 mg of sucrose, Suc40 = isolNDF + 40 mg of sucrose, Suc60 = isolNDF + 60 mg of sucrose.
[3]Values describing the fit of the quadratic regression equation for sucrose effect: for total gas mL of the slowly fermented fraction, R^2 = 0.33 and RMSE = 1.458; for the kd of the rapidly fermented fraction, R^2 = 0.24 and RMSE = 0.0428. For all other items, R^2 values for the quadratic regression equations were no more than 0.01 greater than those achieved with the linear equations, and RMSE values were greater, equal to, or showed less than 3% decrease for quadratic over linear equations.
[4]Root mean square error (RMSE). Regression equations can be calculated from the least squares means.
[5]kd = fractional rate of fermentation.

cles during fermentation. As to this last point, it is possible that if NDF particles were reduced in size through microbial fermentation such that they passed through the frit of a coarse porosity Gooch crucible (40 to 60 μm nominal maximum pore size) during NDF analysis. They would be accounted as fermented substrate, although they were not.

For the rapidly fermented fraction, gas production increased linearly with increased sucrose inclusion (Table 1; $P < 0.01$), which agrees with the concept that gas yield increases as more substrate is fermented (Blümmel et al., 1997). As with the slow fraction, lag time also increased slightly and linearly ($P < 0.01$). The rate of fermentative gas production from the rapidly fermented fraction tended to decrease linearly as more sucrose was included ($P = 0.07$). As will be discussed below (see Carbon Balance), this might have been due to shifts in fermentation product ratios rather than to changes in rate of sucrose consumption. Little information has been reported on changes in the fermentation rate of rapidly fermented carbohydrate with change in quantity of substrate. Oba and Allen (2003) reported an increase in rate of starch degradation in vivo with increasing inclusion of dietary starch. Increased microbial production of glycogen could possibly decrease rate for the rapidly fermented pool if the glycogen remained in that pool and was metabolized at a slower rate than was free sucrose.

The regression equations describing the effect of sucrose inclusion level on gas production and kinetic measures varied in how well they fit the data for measures where sucrose effects were significant or tended to be significant (Table 1). The linear regressions fit the data very well for total gas production and lag time of the

Table 2. Organic acid production and NDF disappearance at 48 h from the fermentation of sucrose and isolated NDF in the gas production measurement system (Exp. 1)[1]

Item	Substrate[2]				SEM	*P*-value, sucrose effect	
	isolNDF	Suc20	Suc40	Suc60		Linear	Quadratic
Proportion of NDF disappearing	0.752	0.753	0.760	0.731	0.013	0.33	0.25
VFA,[3] mmol	0.221	0.364	0.535	0.691	0.015	<0.01	0.66
Molar proportions							
Acetate	0.671	0.637	0.612	0.604	0.004	<0.01	<0.01
Propionate	0.271	0.265	0.286	0.280	0.007	0.11	0.99
Butyrate	0.059	0.099	0.102	0.116	0.005	<0.01	0.01
Branched chain VFA, mmol	–0.001	–0.003	–0.007	–0.008	0.001	<0.01	0.59
Valerate, mmol	0.002	0.004	0.007	0.006	0.001	<0.01	0.11

[1]Values presented as least squares means.
[2]isolNDF = isolated NDF alone, Suc20 = isolNDF + 20 mg of sucrose, Suc40 = isolNDF + 40 mg of sucrose, Suc60 = isolNDF + 60 mg of sucrose.
[3]VFA = acetate + propionate + butyrate.

rapidly fermented fraction, as well as the lag time of the slowly fermented fraction, as evidenced by R^2 values of ≥0.83. The fit of the equations to the remaining measures was not as good. For total gas production for the slowly fermented fraction, although the linear effect of sucrose was significant ($P = 0.02$) and the quadratic effect only tended to be significant ($P = 0.08$), the 0.10 greater R^2 of the quadratic equation indicated that it gave a better fit than the linear equation. This data offers a case in point where the significance of the pattern of a treatment effect does not necessarily equate to an assurance that the significant pattern describes the data well. In terms of the biological significance of the R^2 values, when the sucrose effect approached or reached statistical significance, sucrose inclusion level or some factor correlated with it accounted for 20 to 99% of the variation in responses measured in this experiment.

Total VFA (mmol; sum of acetate, propionate, and butyrate) and valerate at 48 h increased linearly with increasing sucrose ($P < 0.01$ for both; Table 2). However, the molar proportions of individual VFA differed as to whether they increased or decreased with increasing sucrose inclusion (Table 2). The production of branched chain VFA decreased linearly as sucrose increased ($P < 0.01$). The negative values for these products of protein degradation likely reflect a greater utilization for protein (Russell and Sniffen, 1984) or fatty acid synthesis (Allison et al., 1962) than occurred in the fermentation blanks, which contained no substrate to drive microbial growth other than the rumen inoculum. Decreases in ruminal branched chain VFA concentrations have been reported with inclusion of sucrose in the diets of lactating cows (Sannes et al., 2002).

Experiment 2. Fermentation Products

Selection of Correction Values. Selection of appropriate correction values was a critical issue that needed to be addressed for the calculation of fermentation products from sucrose. Fermentation blanks are included for use in correcting the values from the treatments for background measurements that are not directly a result of the treatments. Blanks contain no added substrate, but do contain inoculum and OM in the medium (true protein, peptides, AA) that can be fermented. Values for fermentation products or residual substrate that are measured on fermentation blanks are commonly subtracted from values of fermentations containing substrate (treatments) to assess production of fermentation products attributable to the added substrate. However, this approach is not always sound because of differences in the behavior of various analytes between fermentation blanks and treatments. Analytes that are affected by anabolic and catabolic processes, such as glycogen or TCACP (used as a measure of MCP), are cases in point. Substantial amounts of TCACP and glycogen are introduced with the inoculum at 0 h. In the fermentation blank, both decline with time as a result of fermentation, microbial death, lysis, and recycling in a culture with limited substrate (TCACP example, Figure 1a). In contrast, values for actively growing cultures in vessels with added substrate increase, probably to the point of substrate limitation, before they begin to decline. Fermentation blank values representative of dying cultures are not an appropriate correction for growing cultures and serve only to increasingly inflate TCACP or glycogen values as the fermentation proceeds (e.g., TCACP in Figure 1b). The fermentation blanks also do not account for the TCACP in substrate present at 0 h (the difference between treatments and blanks at 0 h) or for glycogen produced from substrate at 0 h.

Correction values based on 0 h inputs appear to be the most appropriate adjustments for analytes that are introduced in substantial quantities at 0 h, are produced anabolically in growing cultures, and are also subject to degradation. This correction method was used for MCP, for protein degradation products (branched chain VFA), and glycogen in this study. Valerate values were also corrected by this method because valerate may be formed by degradation of carbohydrate and organic acids (Marounek et al., 1989) as well as from protein (Hungate, 1966), potentially giving different responses or production from different substrates in the carbohydrate-restricted fermentation blanks compared with the sample fermentations. Correction of TCACP values at all hours by subtracting the 0-h values for each treatment takes into account the amount of analyte introduced with the inoculum, medium, and substrate (TCACP example, Figure 1c). If the amount of substrate is variable, correction will involve separate adjustments for the 0-h fermentation blank and for the contribution of substrate. The need for separate adjustments will depend on the variability in the amount of substrate added to the fermentation vessels; with little variation, adjustment for the average 0-h treatment values should suffice. For glycogen, because it may be produced rapidly from the substrate and is introduced with the inoculum, subtraction of the 0-h fermentation blank values from all hours allows accounting of the initial production from added substrate (this approach for glycogen would not work with starch-containing substrates). That the values of glycogen and TCACP can increase, decline, or become negative emphasizes that they are the net of synthesis and degradation, and the latter will eventually predominate in batch cultures.

Analytes that are produced by catabolism, and not the net of catabolism and anabolism, may be suitably corrected by subtraction of the values for the fermentation blank at each sampling point. For instance, for VFA such as acetate, concentrations in both the blanks and treated vessels increase with time (Figure 2a). The increase in the fermentation blanks comes from fermentation of OM supplied by the inoculum and medium, which is also present in the treated vessels. Subtracting the value of the fermentation blank at each hour allows accounting of the VFA produced from the inoculum and

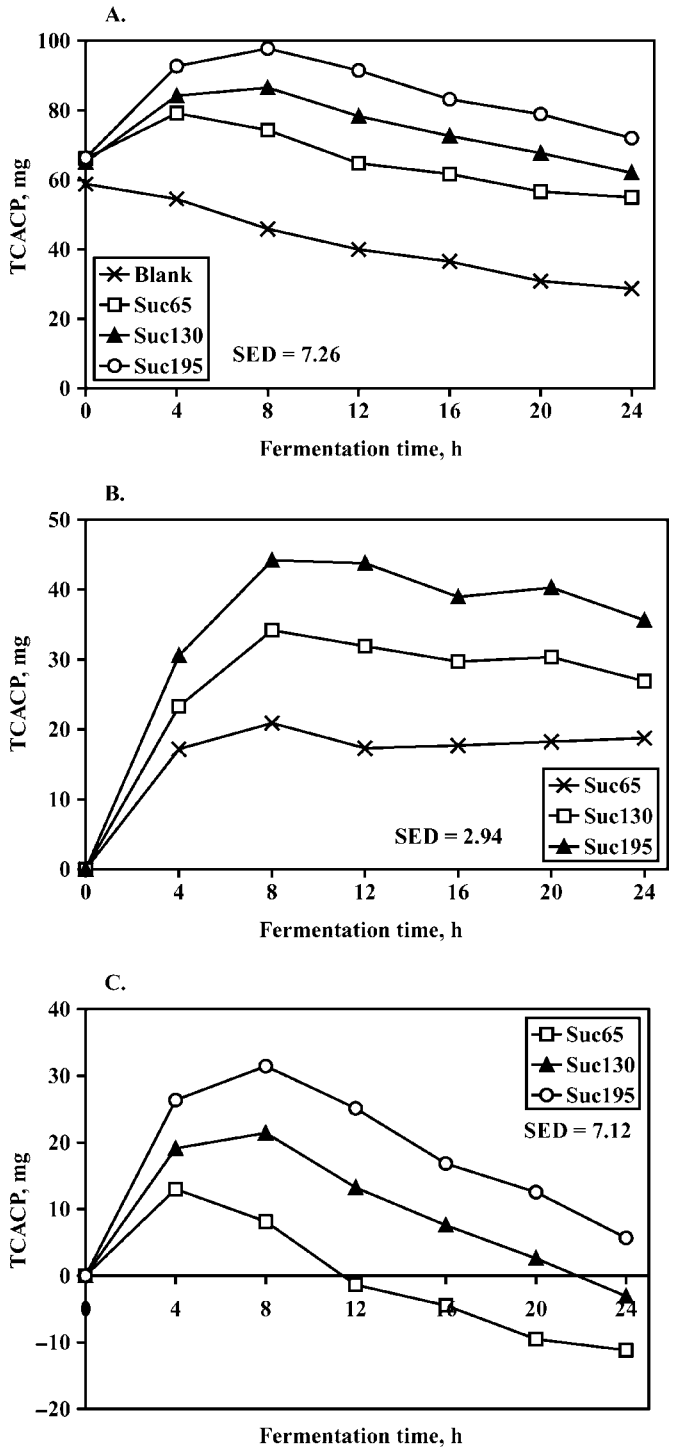

Figure 1. Values (least squares means) for trichloroacetic acetate-precipitated CP (TCACP) from Exp. 2. a) Uncorrected data, b) data corrected for values of fermentation blanks at each hour, and c) data with values of each treatment corrected for its value at hour zero (equivalent to microbial CP). Blank = fermentation blank, Suc65 = 130 mg of isolNDF + 65 mg of sucrose, Suc130 = 130 mg of isolNDF + 130 mg of sucrose, and Suc195 = 130 mg of isolNDF + 195 mg of sucrose.

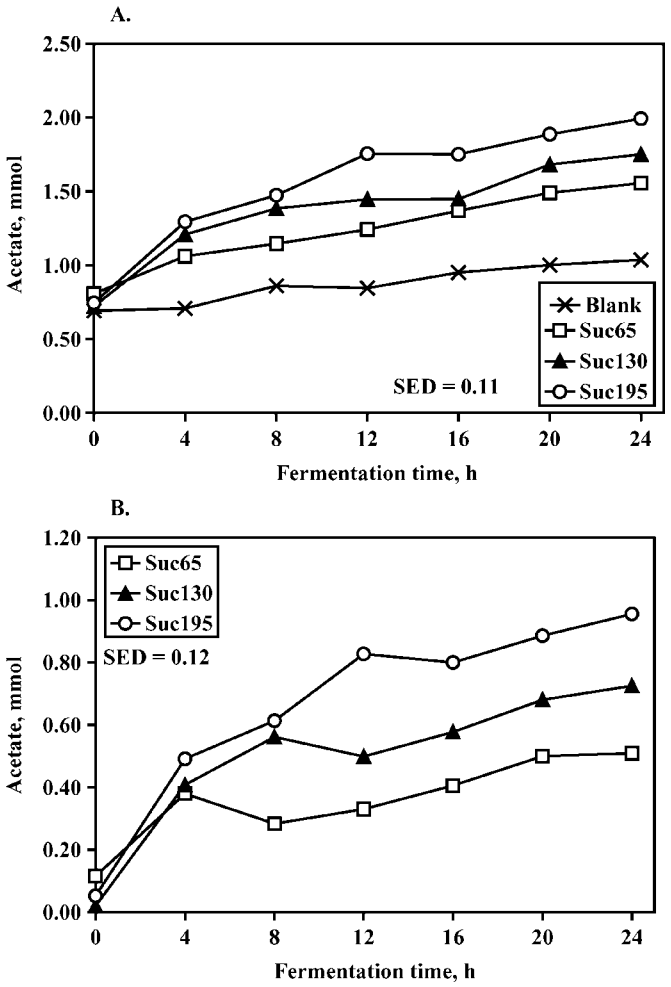

Figure 2. Values (least squares means) for acetic acid in medium from Exp. 2. a) Uncorrected data, and b) data corrected for values of fermentation blanks at each hour. Blank = fermentation blank, Suc65 = 130 mg of isolNDF + 65 mg of sucrose, Suc130 = 130 mg of isolNDF + 130 mg of sucrose, Suc195 = 130 mg of isolNDF + 195 mg of sucrose.

medium exclusive of the deliberately added substrate in the treatments (Figure 2b). This approach seemed appropriate for correction of VFA (acetate, propionate, butyrate) that are primarily derived from carbohydrate, lactate, residual NDF, fructose, glucose, and predicted fermentation gases, but there is a caveat. In the current study, correction for VFA and estimated gas production in fermentation blanks was used to correct for mass of C in inoculum- and medium-derived fermented materials. This approach assumes equal fermentability of the endogenous substrates but does not ensure that the products of the fermented C were the same in fermentations with and without added substrate.

Fermentation blanks in this experiment were estimated to contain 52 to 56 mg of material that fermented during the course of 24 h (hexose basis, based on theoretical stoichiometrics; Hungate, 1966). That amount is not insignificant relative to the amount of substrate

in the treatments and may be common in in vitro fermentations using similar proportions of inoculum, medium, and substrate. The assumption that underlies batch culture in vitro systems that use ruminal inoculum is that the fermentable material in the inoculum and medium ferments at the same rate and to the same products in both the fermentation blanks and treatments alike. That assumption may or may not be reasonable for some fermentation products. It is warranted for the investigator to assess how a fermentation product behaves in the fermentation blank relative to the treatments and then choose an appropriate correction value to assess formation of the product.

Substrate Disappearance. Sucrose disappeared rapidly from the media, with none detected in the 0-h sample. Glucose and fructose detected in the media at 0 h were presumed to be hydrolysis products of sucrose formed during sample collection and work-up. Amounts of both hexoses increased linearly at 0 h as sucrose increased (glucose: 30.7, 48.6, and 75.7 mg, linear P = 0.04, SED = 8.7; fructose: 20.0, 44.6, and 66.0 mg, linear P = 0.01, SED = 5.2; for Suc65, Suc130, and Suc195, respectively). These monosaccharides together accounted for only 74, 68, and 69% of the sucrose in Suc65, Suc130, and Suc195, respectively. The rapid disappearance of substrate agreed with the reports of extremely rapid ruminal hydrolysis of sucrose (1,200 to 1,404%/h) and fermentation of glucose (422 to 738%/h; Weisbjerg et al., 1998). Only trace amounts of fructose were detected in the media by 4 h. The concentrations of glucose found during later sampling hours could be the result of microbial lysis and hydrolysis of glycogen to release glucose. The glucose concentrations (0.24, 0.44, and 0.64 m*M* for Suc65, Suc130, and Suc195, respectively; SED = 0.24) noted for h 12 through 24 are greater than those measured in the rumen (0.06 to 0.08 m*M*; Lynd et al., 2002).

Fermentation Products. Maximal amounts of microbial glycogen were detected at the 0 and 4 h (Suc65 and Suc130) and 4 h (Suc 195) sampling times in the 2 fermentation runs (0.26, 3.84, and 4.30 mg were detected glycogen maxima for Suc65, Suc130, and Suc195, respectively; SED = 2.38). Glycogen values for Suc65 and Suc130 were numerically greater (P = 0.29) than those for the fermentation blank at 0 h, indicating immediate uptake and use of substrate by metabolically active cells, and complements the data on disappearance of sucrose at 0 h. The glycogen content of all treatments declined after their peaks and converged in later hours. There was no detected effect of treatment on glycogen at the maximal detected values or at the detected MCP maxima (P > 0.20; Table 3), no doubt in part due to the variability of the data.

Organic acid production reached its maxima at 24 h, with the exceptions of lactate and butyrate; butyrate appeared to plateau after 4, 8, and 12 h of fermentation for Suc65, Suc130, and Suc195, respectively (data not shown). Total amount of organic acids produced increased (P = 0.01) linearly with sucrose treatment at 24 h and tended to increase (P = 0.07) linearly with sucrose treatment at the times at which MCP maxima were detected (Table 3). The major portion of the organic acids was produced by the time that MCP maxima were detected; this amount did not differ from the amount of organic acids measured at 24 h (effect of time at MCP detected maxima vs. 24 h, P = 0.39), likely due to variability of the data.

Maximum lactate concentrations were detected at 0 h (Suc65), or at 0 or 4 h (Suc130), or at 4 h (Suc195). Maximal lactate increased linearly with increasing sucrose (0.030, 0.060, and 0.154 mmol for Suc65, Suc130, and Suc195, respectively; linear effect of sucrose P = 0.01; quadratic effect of sucrose P = 0.10; SED = 0.013). The appearance of lactate in the 0-h samples suggests the rapidity with which sucrose was fermented. The yield of lactate tended to increase linearly with sucrose treatment (0.457, 0.461, and 0.791 mmol/g sucrose for Suc65, Suc130, and Suc195, respectively; linear effect of sucrose, P = 0.09; SED = 0.107). Lactate declined to undetectable concentrations by 8 h.

The molar proportions of acetate tended to decrease linearly (P = 0.13), but those of propionate and butyrate did not differ ($P \geq 0.30$) across sucrose treatments at the point of maximum detected MCP, though values for propionate and butyrate increased numerically as the amount of sucrose substrate increased (Table 3). At 24 h, these patterns of response became or tended to become significant, changing linearly with increasing sucrose treatment (linear effect of sucrose for acetate, P = 0.02; for propionate, P = 0.06; and for butyrate, P = 0.11; Table 3). This change in molar proportions of VFA suggests a VFA contribution from the fermentation of isolNDF by 24 h, even though the change in total millimoles of organic acids was not significant in that time. Greater ruminal molar proportions of acetate and less of propionate were reported for cows fed diets containing more fiber compared with cows on lower fiber/higher concentrate diets (Davis, 1967; Weimer et al., 1999). More extensive fermentation of isolNDF at lower sucrose inclusion levels could achieve the change in molar proportions noted and would be in agreement with the lesser lag time at lower sucrose inclusion for the slowly fermented fraction (Exp. 1). Alternatively, there is potential contribution of lactate catabolism to the shift in VFA ratios, but this was not assessed in this experiment.

Branched chain VFA and valerate concentrations did not differ among treatments over the course of the entire fermentation (P > 0.42) or at 24 h (P > 0.84), nor were there linear or quadratic effects of sucrose at 24 h (P > 0.62), or an interaction of time and treatment (P > 0.75). The responses at 24 h differ from the 48-h values from Exp. 1, likely reflecting a greater extent of fermentation of NDF in Exp. 1 as well as the use of 48-h fermentation blanks for correction, rather than for 0-h contributions from the inoculum.

Predicted production of CO_2 and CH_4 increased with increasing sucrose treatment (Table 3). Carbon dioxide

Table 3. Products from the in vitro fermentation of sucrose and isolated NDF (Exp. 2)[1]

Item	Substrate[2]			SED	*P*-value, sucrose effect	
	Suc65	Suc130	Suc195		Linear	Quadratic
At detected MCP maxima						
Microbial CP maxima,[3] h	4, 4	4, 8	8, 8	—	—	—
Microbial CP, mg	13.0	22.3	31.4	2.27	0.02	0.98
Total organic acid,[4] mmol	0.649	1.021	1.214	0.163	0.07	0.59
Organic acid molar proportion						
Acetate	0.582	0.539	0.494	0.036	0.13	0.98
Propionate	0.294	0.316	0.374	0.058	0.31	0.75
Butyrate	0.117	0.130	0.133	0.030	0.65	0.87
Lactate	0.003	0.008	0.000	0.006	0.61	0.33
Glycogen, mg	–1.46	–3.35	–5.15	5.36	0.56	0.99
CO_2,[5] mmol	0.347	0.568	0.667	0.126	0.13	0.64
CH_4,[5] mmol	0.180	0.276	0.282	0.082	0.34	0.58
Hexose utilized,[5] mmol	0.360	0.575	0.690	0.106	0.09	0.64
At 24 h of fermentation						
Microbial CP, mg	–11.18	–3.07	5.67	0.467	<0.01	0.52
Total organic acid,[4] mmol	0.825	1.262	1.709	0.085	0.01	0.96
Organic acid molar proportion						
Acetate	0.617	0.574	0.559	0.009	0.02	0.21
Propionate	0.289	0.326	0.329	0.010	0.06	0.20
Butyrate	0.093	0.100	0.112	0.007	0.11	0.74
Lactate	ND[6]	ND	ND	—	—	—
Glycogen, mg	–12.4	–10.8	–12.2	0.90	0.84	0.20
CO_2,[5] mmol	0.430	0.655	0.905	0.047	0.01	0.79
CH_4,[5] mmol	0.234	0.323	0.433	0.029	0.02	0.73
Hexose utilized,[5] mmol	0.451	0.694	0.950	0.046	0.01	0.90

[1]Values presented as least squares means.
[2]Suc65 = 130 mg of isolNDF + 65 mg of sucrose, Suc130 = 130 mg of isolNDF + 130 mg of sucrose, Suc195 = 130 mg of isolNDF + 195 mg of sucrose.
[3]Hours of detected MCP maxima for fermentation runs 1 and 2.
[4]Total organic acid = acetate + propionate + butyrate + lactate.
[5]Predicted from theoretical stoichiometrics based on organic acid production (Hungate, 1966).
[6]ND = not detected.

production tended to increase linearly ($P < 0.13$) with increasing sucrose treatment at maximum detected MCP production, whereas there was only a numerical increase in CH_4 at that time ($P = 0.34$). Production of both gases increased linearly (CO_2, $P = 0.01$; CH_4, $P = 0.02$) with increasing sucrose treatment at 24 h.

The pH decreased through 24 h for all treatments. As sucrose inclusion increased, minimum pH decreased linearly (6.28, 6.14, and 5.94 for Suc65, Suc130, and Suc195, respectively; linear effect of sucrose, $P = 0.04$). No pH reading was below 6.2 by the time of maximum detected MCP.

Microbial CP production is described by curves that peak and then decline steadily (Figure 1c). The temporal pattern for MCP reflects the early peak in MCP from fermentation of sucrose and the subsequent decline of the microbial mass through death, lysis, and recycling when substrate became limiting or microbial growth on available substrate was low. It appears that production of MCP from fermentation of isolNDF was not sufficient to stem the decline in MCP during the 24-h fermentation. There was no interaction of time × treatment for the declining portion of the curve ($P = 0.36$), suggesting that the slopes of the decay curves for sucrose treatments and fermentation blanks did not differ (Figure 1a). For the declining portion of the curves, linear and quadratic terms for fermentation hour were significant ($P < 0.01$); however, addition of the quadratic term for hour and its interaction terms only improved the R^2 from 0.969 to 0.985. This suggests that the linear form of the model adequately described the decline in MCP over time. Evaluation of individual treatments revealed that the quadratic term for time was significant for the fermentation blanks ($P < 0.01$) and Suc65 ($P = 0.05$), but not for the other treatments ($P > 0.28$). The quadratic term describes the apparent plateauing of the MCP curves late in the fermentation (Figure 1a).

Production of MCP increased linearly with increasing sucrose treatment at the time of maximum detected MCP production ($P = 0.02$) and at 24 h ($P < 0.01$; Table 3). Data from the kinetics portion of this study suggest that the slowly fermenting substrate (including isolNDF) did not ferment appreciably until after 8 h, so MCP at and before 8 h should have largely been generated from fermentation of sucrose alone. Yields of MCP per milligram of sucrose were 0.199, 0.171, and 0.161 for Suc65, Suc130, and Suc195, respectively (linear effect of sucrose, $P = 0.24$; SED = 0.023). Assuming a cell protein content of 0.55 g of cell protein/g of cell DM (Madigan et al., 2000), these MCP yields are

Table 4. Carbon in fermentation products as a proportion of carbon from utilized sucrose at time of maximum detected microbial CP production (Exp. 2)[1]

	Substrate[2]				*P*-value, sucrose effect	
Item	Suc65	Suc130	Suc195	SED	Linear	Quadratic
C in products, mg	32.8	51.6	63.5	3.80	0.02	0.40
Product C, mg/mg of fermented sucrose C						
Total product[3]	1.28	0.98	0.81	0.047	0.01	0.23
Microbial CP	0.257	0.215	0.204	0.020	0.12	0.48
Organic acids[4]	0.822	0.602	0.493	0.068	0.04	0.44
Glycogen	–0.025	–0.029	–0.029	0.047	0.93	0.97
CO_2 + CH_4[5]	0.230	0.193	0.147	0.038	0.16	0.90
Hexose C utilized,[5] mg	25.9	41.4	49.7	—	—	—
Sucrose substrate C, mg	27.4	54.7	82.1	—	—	—

[1]Values presented as least squares means.
[2]Suc65 = 130 mg of isolNDF + 65 mg of sucrose, Suc130 = 130 mg of isolNDF + 130 mg of sucrose, Suc195 = 130 mg of isolNDF + 195 mg of sucrose.
[3]Total product = MCP + organic acids + glycogen + CO_2 + CH_4.
[4]Organic acids = acetate + propionate + butyrate + lactate.
[5]Predicted from theoretical stoichiometrics based on organic acid production (Hungate, 1966).

within the range of observed growth yields reported for pure cultures of several species of sugar-fermenting ruminal bacteria (*Streptococcus bovis, Selenomonas ruminantium, Prevotella ruminicola, Megasphaera elsdenii*) grown in continuous culture at high dilution rates (Russell and Baldwin, 1979).

Carbon Balance. Evaluating the conversion of substrate to fermentation products can be problematic as substrates, medium, and inoculum contribute mass to products. An accounting that uses C rather than OM to calculate the digested substrate captured in products reduces, but does not entirely correct for, the interference. As sucrose was increased, the amount of C in fermentation products increased linearly ($P = 0.02$), but the amount of C in products as a proportion of fermented sucrose C declined ($P = 0.01$; Table 4). The proportion of C from utilized sucrose for Suc65 was 1.28; that is 28% more C than possibly supplied by the sucrose substrate at 8 h of fermentation. A value greater than 1.0 indicates uptake of C from nonsucrose sources. Because it is unlikely that fermentation of NDF provided this amount of C at 8 h, and given that fermentable substrate from the medium and inoculum were corrected for by accounting for VFA produced in the fermentation blanks, the probable source of the additional C is AA or peptides from casein hydrolysate in the medium that were incorporated into microbial mass.

The tendency for a decrease in the proportion of C in MCP ($P = 0.12$) and the numeric decrease in MCP yield from sucrose ($P = 0.24$) as sucrose was increased may relate to an increase in the amount of AA and peptides available from the medium relative to microbial N demands driven by the amount of sucrose fermented. The ratios of amino CP from the medium (mg) to sucrose (mg) were 0.88, 0.44, and 0.29 for Suc65, Suc130, and Suc195, respectively. Studies have shown increases in the yield of rumen microbes per unit of carbohydrate as the concentration of AA, peptides, or protein hydrolysate in the medium was increased relative to the amount of carbohydrate (Argyle and Baldwin, 1989; Van Kessel and Russell, 1996). This difference in microbial yield has been related to energy spilling, the increase in nongrowth utilization of carbohydrate by microbial cultures when carbohydrate is in excess of available protein (Van Kessel and Russell, 1996). If specific nutrients (peptides and AA) were not supplemented equivalently among treatments, relative differences in the adequacy of available nutrients could result in different yields of fermentation products (Stokes et al., 1991). This means that the microbial yield may change over a range of fermentable carbohydrate concentrations depending on the relative concentration and perhaps type of nutrient that becomes limiting.

In addition to the effect of amino CP:carbohydrate, the declines in the proportion of C from sucrose in MCP may also be due to catabolic inefficiency. The increase in lactate production with increasing sucrose treatment is in accord with reports that, in some microbial species (*S. bovis,* Russell and Hino, 1985; *S. ruminantium,* Melville et al., 1988), rapid growth at high sugar concentrations results in fermentation product shifts toward lactic acid production, with a resultant decrease in catabolic efficiency (ATP produced per unit hexose consumed) despite an increase in rate of ATP synthesis (ATP produced per unit time). Because increased lactate production also reduces gas yields per unit hexose fermented, a shift in end product ratios toward lactate at high sucrose concentrations (as opposed to a decrease in sucrose consumption rate) could explain the observed decrease in rate of gas production for the rapidly fermented fraction, and thus the predicted rate of fermentation, with increasing sucrose inclusion (Table 1). Both catabolic inefficiency and energy spilling reduce the derivation of energy from carbohydrate fermentation. A decrease in energy available to the microbes could

limit the synthesis of macromolecules including protein (the most energetically demanding of all macromolecular biosyntheses; Stouthamer, 1973), thus limiting the yield of microbial mass and MCP from fermented substrate.

Even with C from MCP excluded from the total C in products, yield of C in products per unit of sucrose utilized still differed among treatments at 8 h (1.03, 0.77, and 0.61 for Suc65, Suc130, and Suc195, respectively; linear effect of sucrose treatment, $P = 0.02$; SED = 0.054). The change in the yields of organic acids were particularly notable (Table 4). This suggests that the yields of fermentation products other than MCP were altered by changing sucrose concentration. Whether this was due to sucrose inclusion alone or to the relative change in the ratio of sucrose to other nutrients utilized by the microbes is open to question. Stokes et al. (1991) reported that changes in the amount of degradable protein relative to the amount of nonfiber carbohydrate altered organic acid production, molar proportions of some organic acids, substrate digestibility, and bacterial efficiency (g bacterial N per kg of digestible DM) when evaluated using feedstuffs and mixed rumen microbes in continuous culture. The greatest bacterial efficiency, total VFA production, and NDF digestion were achieved at the highest level of degradable protein supplementation in that study. Additionally, Aldrich et al. (1993) noted a lower ruminal pH (6.29 vs. 6.40) and greater concentrations of VFA (147.9 vs. 135.6 m*M*) in lactating dairy cattle offered diets high in nonstructural carbohydrates (36 to 38% of DM; predominantly from starch) when a greater vs. lower proportion of dietary protein was ruminally available. In that study, no difference was detected in the amount of OM that was truly digested in the rumen nor in DMI. Combined with the outcome of the current study, these results suggest that the ratio of protein and carbohydrate available to the ruminal microbes may influence the yield of organic acids from fermented carbohydrate.

Previous studies (Argyle and Baldwin, 1989; Van Kessel and Russell, 1996) have shown that the improvement in microbial yield with increasing AA, peptides, or protein hydrolysate in the medium relative to carbohydrate did not show a definitive plateau in response even at the highest protein concentrations. This information, combined with the data of Stokes et al. (1991) on the effect of degradable protein on other fermentation products and substrate digestibility, show the potential for interactions of fermentable carbohydrate and degradable protein to alter the outcome of a fermentation. This raises the question of what concentrations of carbohydrates and degradable protein should be used in vitro if the results are to reflect ruminal fermentation outcomes in vivo. This will be an issue in experiments designed to describe fermentation kinetics and products, and also in comparisons of natural feedstuffs, as they have potential to vary greatly in composition. Will variation in fermentable substrate composition lead to measured differences among feedstuffs that owe much to the proportional effect of components in the medium and that may or may not be representative of how the feed would function as a dietary component in vivo? Evaluation of in vitro fermentation conditions to find those that best emulate the range of physiologic conditions in vivo would seem advised.

The estimate of C in products included the C from MCP but not C from the unaccounted portion of the microbial cell mass. With estimates of microbial cell composition as 55% protein (exclusive of RNA, DNA, and other NPN; Madigan et al., 2000) and 50% C (NRC, 1993), the amount of C associated with microbial cell mass may be underestimated by approximately 70% in our calculations. Inclusion of this additional microbial cell C raises recovery of C from sucrose to approximately 96% for Suc195. However, this adjustment makes the amount of C in products at peak detected MCP production even more in excess of that supplied by sucrose for the other treatments. This necessitates consideration of what the source of the additional C is and what factors we should consider when attempting to perform a C balance with in vitro systems.

In summary, it is common for the proportions of different nonfiber carbohydrates (sugars, starch, soluble fiber, etc.) in ruminant diets to change as inclusion levels of different feedstuffs are modified. However, proportions of other dietary components such as protein fractions, fiber and nonfiber carbohydrates, fats, and minerals may or may not change when these modifications are made, resulting in changing ratios of the dietary fractions. The change in fermentation product yields and kinetics with changing quantities of sucrose noted in the current study and the potential for interactions with components in the medium to have influenced the outcomes indicate a strong need to carefully define and evaluate the run conditions, substrates, media, inoculum source, background corrections, etc. of the fermentation. That information can be used to define how the results are integrated into and applied to our knowledge of ruminant nutrition. The same concerns likely have bearing on in vivo studies.

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